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Letter to the Editor

Molecular Characterization of a Multidrug-Resistant IncF Plasmid Carrying *mcr-3.1* in an *Escherichia coli* Sequence Type 393 Strain of Wastewater Origin

The emergence of multidrug-resistance (MDR) in Enterobacteriaceae is associated with increased global health risks. Strains coharboring mobile colistin resistance genes and other resistance genes are of particular concern because carriage of those genes can lead to pandrug-resistance. In our previous study, we characterized extended-spectrum β-lactamase-producing *Escherichia coli* strains in wastewater collected in Japan [1]. Further analysis revealed that one of those strains, JSWP006, carried *mcr-3.1*, a plasmid-mediated colistin resistance gene originally described in an *E. coli* strain in China [2]. In the present study, we determined that *mcr-3.1* is carried with 15 other resistance genes on a single IncF plasmid in JSWP006, and report the molecular characteristics of this plasmid.

De novo hybrid assembly of JSWP006 using Illumina reads and Nanopore reads was performed as described in the online supplemental material. The hybrid assembly resulted in six contigs (5 102 712 bp, 123 579 bp, 99 519 bp, 5036 bp, 2264 bp, 1313 bp). The second largest contig (123 579 bp) was circular and carried IncF replicons. This plasmid was typed as F-:A1:B1 according to the FAB (FII, FIA, FIB) formula [3]. The third largest contig (99 519 bp) was also circular and contained an FII replicon (F4:A-:B-). The three remaining contigs (all <10 000 bp) seemed to be parts of the chromosome and this was confirmed by long-read-only assembly. The JSWP006 strain contained 16 antibiotic resistance genes (14 complete and 2 truncated genes), including *mcr-3.1* and $bla_{\text{CTX-M-55}}$, and all were on the F-:A1:B1 plasmid. This multidrug-resistant IncF plasmid was named pJSWP006_1 and annotated as described in the online supplemental material.

Figure S1 shows the genetic structure of pJSWP006_1. Plasmid pJSWP006_1 contains an almost identical RepFIA replicon to pRSB107 (the first fully sequenced multi-replicon IncF plasmid with the F1:A1:B1 replicon type) [3], except the RepFIA in pJSWP006_1 is split into two parts in *ccdB* by IS*91*-like insertion sequences. RepFIB is identical in pJSWP006_1 and pRSB107. pJSWP006_1 carries an *oriV-repA4* locus, which has about 98% identity to the corresponding region of RepFII in pRSB107 but lacks the other genes constituting pRSB107 RepFII. Among the *tra* genes essential for F conjugation, pJSWP006_1 carries only four truncated *tra* genes (*traI, traD, traG*, and *traB*). This is consistent with the unsuccessful conjugation attempts (three replicate experiments) using the azide-resistant *E. coli* J53 as the recipient. Besides disrupted *ccdAB*, pJSWP006_1 carries another addiction system, *vagCD*, which may contribute to the maintenance of this plasmid. All 16 resistance genes are clustered in the 64 kb multiresistance region, which is described in detail below.

The genetic environment of *mcr-3.1* on pJSWP006_1 is closely related to that of the IncR plasmid pHN8 [4], except a 22 945 bp composite transposon harboring four antibiotic resistance genes (*qnrS1, floR,* $bla_{\text{CTX-M-55}}$, and *catA2*) is inserted in *tmrB* (Fig. 1A, Fig. 1B, Fig. 1C). This transposon is bracketed by IS*15DI* elements (different from the reference sequence of IS*26* in ISfinder by three nucleotides) and is flanked by 8-bp direct repeats (CTTTCTGA). A BLAST search revealed that each resistance gene is embedded within a genetic context found on other plasmids (pEQ2 for *qnrS1*, pRCADGH-1 for *floR*, and pHNHN21 for *catA2* and $bla_{\text{CTX-M-55}}$). This transposon contains three IS*26* and three IS*15DI* elements. Analysis of target site duplications identified that the 8-bp sequence adjacent to the left-hand inverted repeat of an IS*26* is the reverse complement of the 8-bp sequence adjacent to the right-hand inverted repeat of an IS*15DI*. This implies homologous recombination between IS*26* and IS*15DI* subsequent to IS*26* insertion.

The structure of the resistance region upstream of *mcr-3.1* is shown in Fig. 1B. This region contains a class 1 integron with the trimethoprim resistance gene cassette *dfrA17* (In987), a Tn*6026*-like structure, and a Tn*10*-like structure. Tn*6026* consists of two transposons, Tn*6029* and Tn*4352*, and contains $bla_{\text{TEM-1B}}$, *sul2, strA, strB*, and *aphA1*. However, the Tn*6026*-like structure in the present study is different from the original Tn*6026* in that the $bla_{\text{TEM-1B}}$-containing region between two IS*26* is inverted. This may have been caused by homologous recombination between two IS*26* elements in opposite orientations. Moreover, one of the IS*26* elements was deleted, probably caused by intramolecular transposition in *cis* of IS*1R*, as IS*1* family members are considered to use replicative transposition [5]. Tn*10* carries IS*10*-L, *jemA, jemB, jemC, tetR, tet*(B), *tetC, tetD*, and IS*10*-R. However, IS*10*-L, *jemA*, and the 5' part of *jemB* are deleted in the Tn*10*-like structure described in the present study. Furthermore, an IS*1R*-like element and IS*26* are inserted in IS*10*-R, generating direct repeats of CCGAATTA and CATCAAAC, respectively. The structure of the resistance region downstream of *mcr-3.1* is shown in Fig. 1C. The *mef*(B) gene was found to be disrupted by IS*26* and is within the context of IS*26*-Δ*mef*(B)-*yusZ*-Δ*yqkA*. The *erm*(B) gene is within the genetic context found in the chromosome of *E. coli* strain MS8345. Interestingly, this region is duplicated in the chromosome of MS8345, although only one copy is present on pJSWP006_1.

pJSWP006_1 carries 10 copies of intact IS*26*, five copies of intact IS*15DI*, and three copies of truncated IS*26*. This indicates that translocatable units (circular molecules consisting of a DNA segment and one copy of IS*26*) are readily generated via replicative transposition in *cis* of IS*26* or by homologous recombination between two IS*26* elements, facilitating the movement of resistance genes [5,6]. A recent study showed that an intermediate circle carrying *mcr-3.1* was generated via homologous

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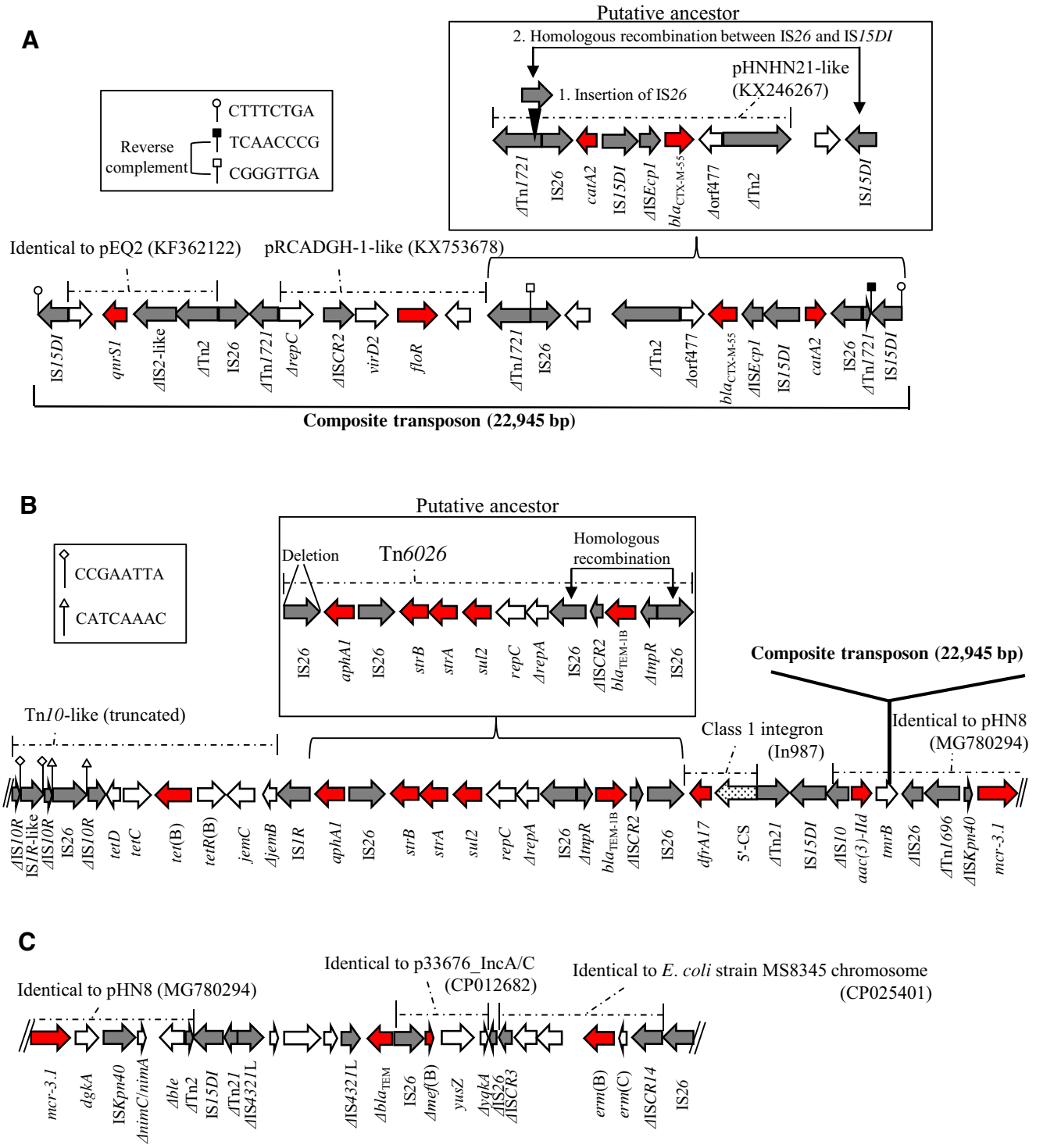

Fig. 1. (A) IS*15DI*-flanked composite transposon carrying four antibiotic resistance genes (*qnrS1, floR,* $bla_{CTX\text{-}M\text{-}55}$, and *catA2*), which is inserted in *tmrB* as shown in (B). (B) Structure of the resistance region upstream of *mcr-3.1*. (C) Structure of the resistance region downstream of *mcr-3.1*. Circles, squares (filled or empty), diamonds, and triangles on short stalks are used to indicate target site duplications. Red arrows indicate antibiotic resistance genes, gray arrows indicate mobile elements, a dotted arrow indicates a 5'-conserved segment (5'-CS) of a class 1 integron, and white arrows indicate other genes.

recombination between a truncated IS*26* and an intact IS*15DI* [4]. Although pJSWP006_1 was not self-transmissible, highly abundant IS*26* (and IS*15DI*) elements can contribute to the movement and dissemination of resistance genes.

Importantly, JSWP006 was classified as an extraintestinal pathogenic *E. coli* (ExPEC) based on its virulence gene profile, and belongs to D-ST393, which is one of the major pandemic ExPEC clonal lineages (see the online supplemental material for detailed analysis of virulence gene content of JSWP006). Detection of *E. coli* ST393 carrying this multidrug-resistant plasmid in wastewater highlights the need to monitor for antibiotic resistance in the environment.

Nucleotide sequence accession numbers

The sequence data generated by Nanopore sequencing and the annotated sequence of pJSWP006_1 have been deposited in the DDBJ under accession numbers DRA007285 and AP018939.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.06.024.

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Declarations

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Competing interests: None to declare.

Ethical approval: Not required.

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